Exhibit 2

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Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

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Cold Spring Harbor Laboratory Press
1989

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Printed in the United States of America

9 8 7 6 5

Book and cover design by Emily Harste

Cover: The electron micrograph of bacteriophage λ particles stained with uranyl acetate was digitized and assigned false color by computer. (Thomas R. Broker, Louise T. Chow, and James I. Garrels)

Cataloging in Publications data

Sambrook, Joseph
Molecular cloning : a laboratory manual / E.F. Fritsch, T. Maniatis—2nd ed.
p. cm.
Bibliography: p.
Includes index.
ISBN 0-87969-309-6
1. Molecular cloning—Laboratory manuals. 2. Eukaryotic cells--Laboratory manuals. I. Fritsch, Edward F. II. Maniatis, Thomas III. Title.
QH442.2.M26 1987
574.87'3224—dc19 87-35464

All Cold Spring Harbor Laboratory Press publications may be ordered directly from Cold Spring Harbor Laboratory Press, 10 Skyline Drive, Plainview, New York 11803. Phone: 1-800-843-4388. In New York (516) 367-8423. FAX: (516) 367-8432.

3. After homogenization, add sodium lauryl sarcosinate to a final concentration of 0.5% and mix the suspension well. Centrifuge at 5000*g* for 10 minutes at room temperature.

   Sodium lauryl sarcosinate is used as a detergent because SDS is insoluble in concentrated salt solutions.

   The centrifugation step can usually be omitted when processing lysates of cultured cells.

4. Transfer the supernatant (or the lysate of cultured cells) to a fresh tube, and draw it into a hypodermic syringe fitted with a 23-gauge needle. Layer the samples onto a cushion of 5.7 M CsCl, 0.01 M EDTA (pH 7.5) in a clear ultracentrifuge tube. Using a waterproof marker, mark the position of the top of the cushion on the outside of the tube. Use guanidinium thiocyanate homogenization buffer to fill the tubes and to equalize their weights.

   The CsCl/EDTA solution is made in 100-ml batches by dissolving 96.0 g of CsCl in 90 ml of 0.01 M EDTA (pH 7.5) and adding diethyl pyrocarbonate (DEPC) to a final concentration of 0.1%. Allow the solution to stand for 30 minutes, and then autoclave for 20 minutes at 15 lb/sq. in. on liquid cycle. When the solution has cooled, adjust the volume to 100 ml with DEPC-treated water (see page 7.4).

   Caution: DEPC is suspected to be a carcinogen and should be handled with care.

   For convenience in later steps, the tubes should be labeled on the bottom.

5. Centrifuge at 20°C for the time and at the speed indicated in the table below.

   A swinging-bucket rotor is preferred to a fixed-angle rotor because the RNA is deposited at the bottom of the centrifuge tube rather than along the wall (where it comes into contact with the cell lysate).

   Turn off the centrifuge brake before decelerating the rotor to prevent disturbing the contents of the tube.

Rotor	Volume of CsCl/ EDTA cushion (ml)	Volume of homogenate (ml)	Time (hours)	Speed (rpm)
SW60 ($7/16'' \times 2\frac{3}{8}''$)	1.2	3.1	12	40,000
SW40 ($9/16'' \times 3\frac{1}{2}''$)	3.5	9.7	24	32,000
SW28 ($1\frac{1}{2}'' \times 3\frac{1}{2}''$)	12.0	26.5	26	23,500

6. Take care not to disturb the gradients when removing the tubes from the centrifuge. Draw a line on the outside of each tube approximately 0.5 cm from the bottom. Using a pasteur pipette, carefully remove the fluid above the level of the cushion (upper mark on the outside of the tube). This part of the gradient contains the viscous cellular DNA, which is usually visible as a white band. With a fresh pipette, remove the fluid above the lower mark on the outside of the tube.